Quantitative proteome analysis of pluripotent cells by iTRAQ mass tagging reveals post-transcriptional regulation of proteins required for ES cell self-renewal.

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Public Summary:

Scientific Abstract:
Embryonic stem cells and embryonal carcinoma cells share two key characteristics: pluripotency (the ability to differentiate into endoderm, ectoderm, and mesoderm) and self-renewal (the ability to grow without change in an untransformed, euploid state). Much has been done to identify and characterize transcription factors that are necessary or sufficient to maintain these characteristics. Oct-4 and Nanog are necessary to maintain pluripotency; they are down-regulated at the mRNA level by differentiation. There may be additional regulatory genes whose mRNA levels are unchanged but whose proteins are destabilized during differentiation. We generated proteome-wide, quantitative profiles of ES and embryonal carcinoma cells during differentiation, replicating a microarray-based study by Aiba et al. (Aiba, K., Sharov, A. A., Carter, M. G., Foroni, C., Vescovi, A. L., and Ko, M. S. (2006) Defining a developmental path to neural fate by global expression profiling of mouse embryonic stem cells and adult neural stem/progenitor cells. Stem Cells 24, 889-895) who triggered differentiation by treatment with 1 muM all-trans-retinoic acid. We identified several proteins whose levels decreased during differentiation in both cell types but whose mRNA levels were unchanged. We confirmed several of these cases by RT-PCR and Western blot. Racgap1 (also known as mgcRacgap) was particularly interesting because it is required for viability of preimplantation embryos and hematopoietic stem cells, and it is also required for differentiation. To confirm our observation that RACGAP-1 declines during retinoic acid-mediated differentiation, we used multiple reaction monitoring, a targeted mass spectrometry-based quantitation method, and determined that RACGAP-1 levels decline by half during retinoic acid-mediated differentiation. We knocked down Racgap-1 mRNA levels using a panel of five shRNAs. This resulted in a loss of self-renewal that correlated with the level of knockdown. We conclude that RACGAP-1 is post-transcriptionally regulated during blastocyst development to enable differentiation by inhibiting ES cell self-renewal.